IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	**Michael Meyrick Burrell and Stephen Andrew Coates**
Serial No.:	**08/192,493**
Filed:	**February 7, 1994**
Art Unit:	**1804**
For:	**MODIFICATION OF STARCH PRODUCTION**
Examiner:	**Charles C.P. Rories**

DECLARATION UNDER RULE 37 CFR 1.132

I, **Michael Meyrick BURRELL**, of 20 Histon Road, Cottenham, Cambridge, England, **DO SOLEMNLY** and **SINCERELY DECLARE** as follows:

1. I am the **Michael Meyrick Burrell** who is named as being an Applicant in respect of United States Patent Application Serial No. 08/192,493.

2. I am a scholar of Cambridge University and hold a PhD in Plant Biochemistry and a Degree in Natural Sciences with honours in Botany.

3. I initiated and supervised the conduct of the experiment now to be related.

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4. The coding sequence for the wheat homologue of *waxy* was fused to the high molecular weight glutenin promoter of wheat to produce a chimaeric gene which would be expressed in developing endosperm. A hybrid maize was transformed with this construct using the particle bombardment method and plants were regenerated to produce seed by outcrossing.

5. Analysis of single seeds taken from six independent transformants is shown below. Starch was isolated and the starch granule bound proteins separated by gel electrophoresis. The 60kDa waxy protein is clearly absent from line 3 (Figure 1). The starch from this line clearly has a much reduced amylose content (Figure 2).

Fig.1.

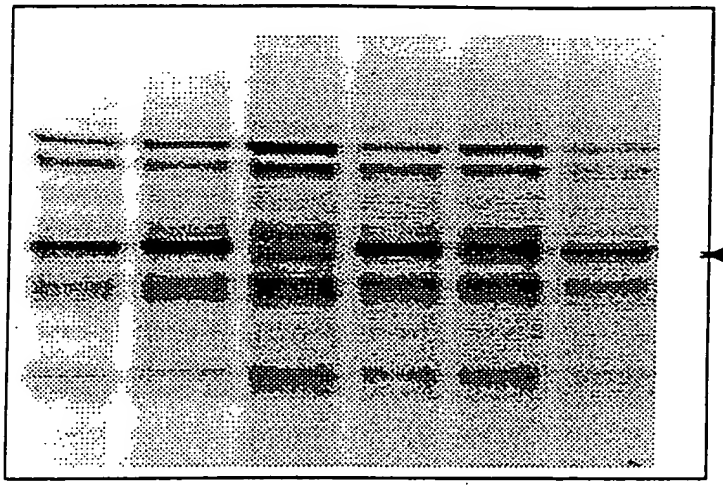
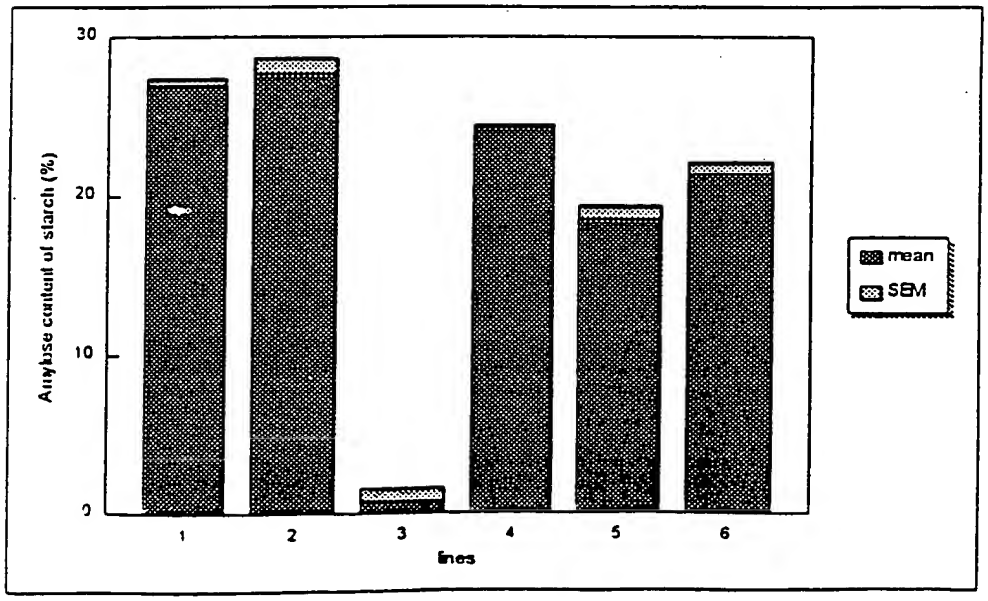


Fig.2.



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6. The seed from the progeny of plants obtained in the transformation procedure were divided into two populations. One population contained plants that showed no clear 60kDa waxy protein product and one population contained segregants that had the 60kDa waxy protein. A third population of untransformed controls were also included in the analysis. The results (table below) clearly show the somewhat surprising result that the absence of the waxy protein is associated with no detectable amylose and no change in starch content.

	Starch Content umol/gfwt		**% amylose**		
Grain analysed	**Mean**	**S.E.M.**	**Mean**	**S.E.M.**	**Number of grain analysed**
Control line grain	91.30593	13.83537	19.28946	1.834417	5
Progeny grain lacking the product of the waxy gene	105.9155	11.64986	nd*	nd*	15
Progeny grain expressing the product of the waxy gene	92.26848	8.171925	21.92222	0.71156	16

***nd = not detected**

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code,

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and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Signature: Michael O. Russell

Date: 22nd June 1996

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael Meyrick Burrell and Keith Stuart Blundy
Serial No.:	08/284,199
Filed:	August 2, 1995
Art Unit:	1804
For:	MODIFICATION OF PLANT METABOLISM
Examiner:	D. Fox

DECLARATION UNDER RULE 37 CFR 1.132

I, Michael Meyrick Burrell, of 20 Histon Road, Cottenham, Cambridge, England, DO SOLEMNLY and SINCERELY DECLARE as follows:

1. I am the Michael Meyrick Burrell who is named as being an Applicant in respect of United States Patent Application Serial No. 08/284,199.

2. I am a scholar of Cambridge University and hold a PhD in Plant Biochemistry and a Degree in Natural Sciences with honours in Botany.

3. I initiated and supervised the conduct of the experimental procedures now to be related.

4. According to a first experimental procedure a chimaeric gene was constructed which contained the

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antisense coding sequence for potato acid invertase and the antisense coding sequence for potato sucrose phosphate synthase (SPS). To each of these two sequences was attached a patatin promoter, to ensure potato tuber specific expression of the deoxyribonucleic acid, and a nos terminator. Potato plants were transformed with this chimaeric gene containing the two sequences. The transformed potato plants were grown in a glasshouse alongside non-transformed control potato plants and tubers were harvested from both populations. Sugars were recovered from the harvested tubers by aqueous extraction after killing in boiling 80% ethanol. Sucrose, glucose and fructose were then determined enzymatically as described by Morrell, S. and ap Rees, T. (1986) Phytochemistry 25 : 1579-1585. All transformed plants analysed were shown by polymerase chain analysis to contain the introduced gene. The table below shows, for the tubers of the test and control populations, the mean values of the sugars and the ratio of sucrose to reducing sugar (glucose plus fructose).

	μmoles/g fr. wt.			
	Glucose	Fructose	Sucrose	Ratio of sucrose/glucose + fructose
Control	20.4	11.9	10.7	0.33
Antisense Invertase/SPS	7.8	2.2	9.8	1.44
Least significant difference	14.02	7.29	4.63	1.09

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5. The statistical computer program Genstat was used to analyse the results from the first experimental procedure. From this analysis calculation was made of the difference that would need to be obtained between the controls and the transformed test population for a significant result. In respect of fructose and the ratio of sucrose to reducing sugar the differences between the control values and the values for the transformed population are greater than the calculated respective least significant differences. Therefore the results for fructose and the ratio of sucrose to reducing sugar are significant at the Fisher P value of 0.05. For glucose the result is significant at a Fisher P value of 0.10.

6. These statistically significant results for the transformed potato plants are advantageous in respect of certain forms of potato processing. Moreover, these results were not accompanied by any deleterious phenotypic effects in the transformed potato plants.

7. According to a second experimental procedure, two chimaeric genes were constructed. The first chimaeric gene contained the potato sucrose synthase coding sequence in the sense orientation attached to a patatin promoter and the second chimaeric gene contained the same coding sequence in the antisense direction and a patatin promoter. Each of these chimaeric genes included a nos terminator. In other

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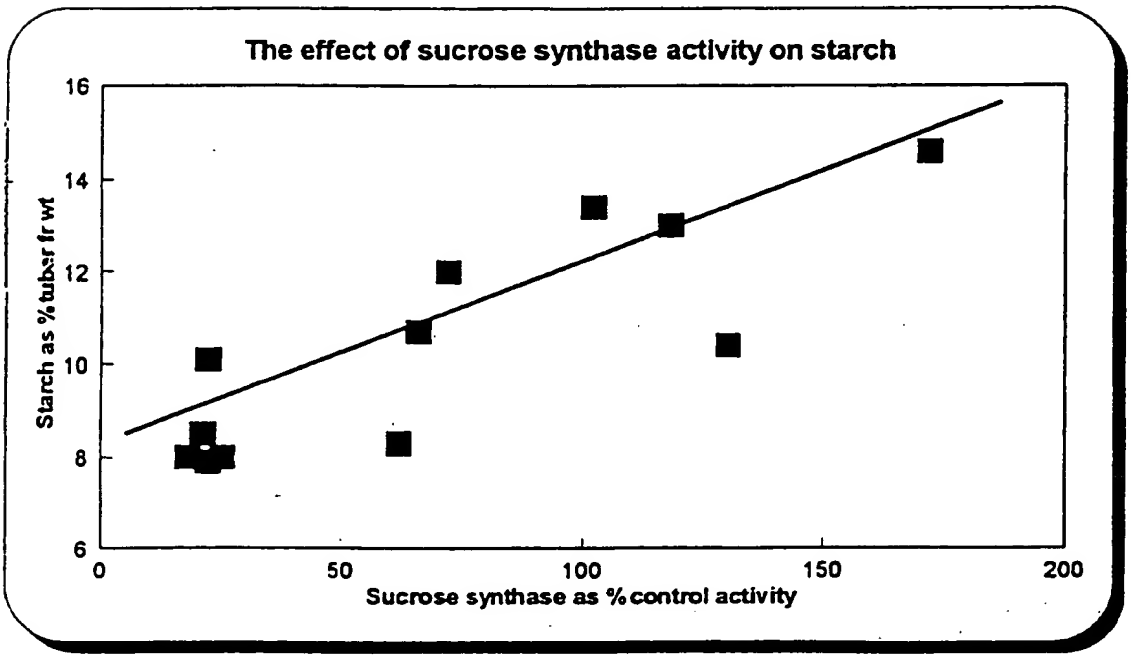
words, the two chimaeric genes were identical except for the orientation of the coding sequence. Potato plants, a proportion of which were transformed with the first chimaeric gene and the remainder of which were transformed with the second chimaeric gene, were grown in the field alongside non-transformed control plants, and tubers from all three of these populations were analysed for their starch content and the activity of sucrose synthase. The values of sucrose synthase activity were then expressed as a percentage of the value thereof in the control plants and plotted against the values for starch content (as shown below). A line of best fit was calculated from the points and gives a significant correlation of 0.58.



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8. It is thus clearly shown that there was a significant correlation between sucrose synthase activity and starch content in the transformed potato tubers. This relationship obtains whether the chimaeric gene contains the coding sequence in the sense or the antisense direction. Therefore, despite variation between individual transgenic plants, the result is predictable and consistent. The result is also commercially important since the starch content required in the tuber for different processing varies.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Signature: Michael H. Bevell

Date: 25th February 1997.